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POSTER ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

Molecular Mechanism of Action of Glucocorticoids in Lymphoma Therapy

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Prednisone is an anti-inflammatory glucocorticoid (GC) that is cytotoxic for normal and malignant B cells and, on this basis, has long been included in combination chemotherapies to treat aggressive B-cell lymphomas, including diffuse large B cell lymphoma (DLBCL) and Burkitt lymphoma (BL). GCs act by binding to the glucocorticoid receptor (GR; *NR3C1*), a ligand-induced transcription factor. The transcriptional response to GCs can vary significantly due to the variation in expression levels of GR cofactors and chromatin landscapes in different cell types. The mechanisms by which GCs kill malignant lymphoma cells are largely unknown, prompting us to search for new targets of GC action with the hypothesis that GCs may inhibit key survival pathways in lymphomas.

To identify genes that synergize or antagonize GC lethality in lymphomas, we performed genome-wide CRISPR-Cas9 screens in the presence and absence of prednisolone, the active metabolite of prednisone. Screens in cell line models of BL and DLBCL (both ABC and GCB subtypes) revealed strong synergy between GC treatment and inactivation of genes encoding components of the B cell receptor signaling pathway, which is required to sustain the viability of these malignant lymphoma cells. With combination of the cleavage under targets and release using nuclease (CUT&RUN) assay and RNA-seq, we identified GR binding to the *LAPTM5* locus at its glucocorticoid response element and AP1 motifs upon GC treatment. GR binding also induced expression of LAPTM5, which negatively regulated BCR signaling by promoting the lysosomal degradation of the BCR.

Conversely, GC induced binding of GR to the *CSK* locus, thereby repressing expression of the non-receptor tyrosine kinase CSK, which antagonizes BCR signaling by phosphorylating an inhibitory tyrosine residue present in all Src-family kinases (SFKs). However, in BCR-dependent aggressive lymphomas, inactivation of CSK paradoxically decreased proximal BCR signaling and induced cell death. By performing quantitative phosphoproteome and ubiquitinome with mass spectrometry, we demonstrated that treatment of lymphoma models with a small molecule inhibitor of CSK kinase activity (CSKi) initially increased constitutive BCR signaling, as expected, but then triggered exuberant ubiquitination of the LYN, HCK and BLK, leading to their proteasomal degradation. Consequently, the CSKi blocked BCR-dependent NF-kB activation in ABC DLBCL models and BCR-dependent PI3 kinase activation in models of GCB DLBCL and BL.

In summary, inhibition of oncogenic BCR signaling is a major mode of action for GCs, which have been used empirically for decades to treat lymphomas. GCs restrain the most proximal steps in BCR signaling at the plasma membrane by, on one hand, decreasing BCR abundance, and on the other hand, by decreasing CSK expression, thereby reducing expression of the essential SFKs. Small molecule inhibition of CSK kinase activity potentiated the effect of GCs on oncogenic BCR signaling and strongly synergized with GCs in killing ABC and GCB DLBCL models in vitro and preventing the growth of ABC and GCB DLBCL and patient-derived xenografts, warranting the development of clinical-grade CSK inhibitors for the treatment of these aggressive cancers.

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